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Oviposition preference of *Encarsia bimaculata* and *Eretmocerus* sp. nr. *furuhashii* (Hymenoptera: Aphelinidae), two parasitoids of *Bemisia tabaci* (Hemiptera: Aleyrodidae)

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Abstract: *Encarsia bimaculata* and *Eretmocerus* sp. nr. *furuhashii* are two dominant species of parasitoid parasitizing *Bemisia tabaci* in South China. Oviposition preference of the two species was studied in the laboratory. The results indicated that both the two species of wasps could parasitize all nymphal stages of *B. tabaci*. When given a single instar nymphs of *B. tabaci*, *E. bimaculata* lay more eggs on the 3rd and 4th instar nymphs than on the 1st and 2nd instars, while *Er.* sp. nr. *furuhashii* lay more eggs on the 3rd and 2nd instars than on the 1st and 4th instars. When given all four *B. tabaci* instars simultaneously, *E. bimaculata* decreased the parasitization on the 1st and 2nd instar nymphs but increased the parasitization on the 3rd and 4th instar immatures; whereas *Er.* sp. nr. *furuhashii* increased its parasitization on the 2nd and 3rd instars but reduced oviposition on the 1st and 4th instars. Host plants showed no influence on the oviposition trends of the two wasps. The results suggested that the 3rd and 4th instar nymphs of *B. tabaci* were the most suitable hosts for *E. bimaculata* while the 2nd and 3rd instars were the more suitable for *Er.* sp. nr. *furuhashii*.

Key words: *Encarsia bimaculata*; *Eretmocerus* sp. nr. *furuhashii*; *Bemisia tabaci*; oviposition preference; biological control

1 INTRODUCTION

The sweetpotato whitefly, *Bemisia tabaci* (Gennadius) is a serious pest of agriculture and horticulture in the world (Costa *et al.*, 1993; Brown *et al.*, 1995; Wang and Tsai, 1996). The first record of *B. tabaci* in China was in 1949 (Chou, 1949), but it was not considered as a major pest until the middle 1990s (Ren *et al.*, 2001; Luo *et al.*, 2002; Qiu *et al.*, 2003). The outbreak of *B. tabaci* as a destructive pest in China in the middle 1990s was attributed to the invasion of a new exotic biotype of *B. tabaci*, which was identified as biotype B (Luo *et al.*, 2002; Qiu *et al.*, 2003). By 2003, the *B. tabaci* B biotype has been reported in more than 28 of 34 provinces of China, and its host plants have been recorded more than 280 plant species (Qiu *et al.*, 2001; Zhou *et al.*, 2003; He *et al.*, 2003; Qiu and Ren, 2005).

Chemical control is still the key method in *B. tabaci* management in many countries including China, but more and more pesticides were reported to loss their effectiveness because of the strong ability of *B. tabaci* B biotype in rapidly developing resistance to most of the commercially available insecticides (Prabhaker *et al.*, 1985, 1989; Dittrich *et al.*, 1990; Ishaaya, 1995; Byrne *et al.*, 2003). Therefore, biological control was thought to be an attractive alternative management for *B. tabaci* (Liu and Stansly, 1996; McAuslane and Nguyen, 1996; Gerling *et al.*, 2001; Ren *et al.*, 2001; Qiu *et al.*, 2005).

Among the natural enemies of *B. tabaci*, parasitic aphelinid in two genera, *Encarsia* Föerster and *Eretmocerus* Haldeman, were considered as the most important and effective parasitoids of *B. tabaci* (Gerling *et al.*, 2001). In South China, *Encarsia bimaculata* (Heraty & Polaszek) and *Eretmocerus* sp. nr. *furuhashii* (Rose & Zolnerowich) were two potential species reported from surveys (Ren *et al.*, 2001; Qiu *et al.*, 2004; Qiu and Ren, 2005). Preliminary parasitism investigation in fields indicated that the total parasitism of these two parasitoids on vegetables and ornamentals were averaged 15% - 22% in a year and the highest record amounted up to 87.3% was found in South China during May 2001 and July 2002 (Qiu *et al.*, 2004).

To date, few detail studies about the biology of *E.*

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bimaculata and *Er*. sp. nr. *furuhashii* have been reported. The objective of present study was to determine the oviposition preference of the two parasitoids first giving a single instar and followed giving all four instars of *B*. *tabaci* nymphs, which are expected to reinforce the biological control studies of *B*. *tabaci*.

2 MATERIALS AND METHODS

2.1 Insects and plants

E. *bimaculata* and *Er*. sp. nr. *furuhashii* used in this study were originally collected on hibiscus, *Hibiscus rosa-sinensis* L., in Guangzhou in 2000. *E*. *bimaculata* was identified by Jian Huang (Fujian Agriculture & Forestry University), and *Er*. sp. nr. *furuhashii* was identified by Mike Rose (Montana State University, USA) and Stefan Schmidt (Zoologische Staatssammlung Muenchen, Germany). *B*. *tabaci* used in this study was collected from the experimental farm of South China Agricultural University (SCAU) on hibiscus in April 2000 and its biotype was identified to be“ B ”biotype using methods of RAPD-PCR (de Barro and Driver, 1997) and mt COI (Frohlich *et al*., 1999). Parasitoids and whitefly were maintained on the same host in rearing cages (60 cm × 60 cm × 60 cm) in laboratory for at least 10 generations before used in study.

Four species, collard (*Brassica oleracea* var. *acephala* L., cv. ‘ Georgia LS’), cucumber (*Cucumis sativus* L., cv. ‘ Xiaqing 2’), eggplant (*Solanum melongena* L., cv. ‘ Yuefengzihong’) and tomato (*Lycospersicum esculentum* Mill., cv. ‘ Hongxing 101’), were used as host plants in this study. Plants were grown in 12 cm diameter pots, watered and fertilized when necessary. Plants were used in the experiments at the 6-leaf stage. Leaf hair density of each plant was measured as described by Qiu *et al*. (2005).

All experiments were conducted in air-conditioned incubators (PXY-300Q-A, Keli Experimental Instrument Co., Ltd., China) at 26.0 ± 0.5℃ with 70% – 80% R.H., photoperiod was 14:10 h (L:D) and light intensity was approximately 2 250 lx.

2.2 Oviposition preference of two parasitoid species

2.2.1 Single host instar experiments: For this test only one instar of *B*. *tabaci* nymphs was exposed to the two species of parasitoids at same time. Forty pairs of *B*. *tabaci* adults were introduced into a clip cage (3.0 cm diameter by 1.5 cm high) covered on the underside surface of plant leaf. Whitefly adults were removed after a 4-h oviposition period to assure nymph stage uniformity. When the nymphs developed to the desired stage (1st, 2nd, 3rd or 4th instar), all but 40 nymphs of each instar were removed carefully using an insect pin, and 3 mated females of *E*. *bimaculata* or *Er*. sp. nr. *furuhashii* were introduced into a clip cage to lay eggs for 24 h. Mated females of parasitoids were obtained through confining new emerged females and males of each species in a petri dish for 6 h, respectively. After a 10-day incubation period, whitefly nymphs were examined under a Leica M10 binocular stereomicroscope. The numbers of parasitized nymphs, unparasitized nymphs and emerged adults were recorded. Experiments for each whitefly instar were repeated 3 times and 10 cages were replicated for each time.

2.2.2 Multiple host instar experiments: All four instars of whitefly nymphs were exposed simultaneously to parasitoids. The arrangement was made by confining 40 pairs of whitefly female adults in a clip cage on a leaf 4 times; intervals of infestation were determined based on primary studies of the developmental time of each instar of *B*. *tabaci* under the experimental conditions, generally a 2.5-day interval for the 2nd instar after first infestation, followed by a 3-day interval for the 3rd instar and then a 2-day interval for early 4th instar. After a 2-week development, the 1st to 4th instar whitefly nymphs were available at the same leaf, all but 10 nymphs each instar were removed carefully, and the nymphs of each instar were circled with different indelible color inks. The oviposition of parasitoids and parasitism of whitefly nymphs were examined as described above, and the numbers of parasitized nymphs, unparasitized nymphs and emerged adults of *B*. *tabaci* were recorded. Experiments were repeated 3 times and 10 cages were replicated for each time.

2.3 Data analysis

The parasitisms of *E*. *bimaculata* or *Er*. sp. nr. *furuhashii* on different instar nymphs of *B*. *tabaci* B biotype were calculated with the following formula: $P\% = 100\ P_L/(W_N + P_L)$, where $P\%$ is percentage of parasitism, P_L is the number of parasitoid larvae, W_N is the number of unparasitized nymphs of *B*. *tabaci*, the whiteflies that emerged during the experiment were also calculated as unparasitized.

Differences in percentage parasitism among different instars of the two species of parasitoids were compared using analysis of variance (PROC ANOVA, SAS Institute, 2003). Means were separated using Student-Newman-Kuels Multiple range test at a significant level of $\alpha = 0.05$ (SAS Institute, 2003).

3 RESULTS

Results showed that *E*. *bimaculata* and *Er*. sp.

nr. *furuhashii* could parasitize all nymphal instars of *B. tabaci*，although there were differences in parasitisms among different instars and host plants. All of parasitoid adults emerged from the 4th instar nymphs or“ pupae ”stage of *B. tabaci*，regardless the whitefly instar at the time they were initially parasitized.

The parasitisms of *E. bimaculata* on *B. tabaci* nymphs in single and multiple instar experiments on collard，eggplant，cucumber and tomato were showed in Table 1. To a certain nymphal stage，the oviposition preferences of this wasp on the 4 host plants were consistent，parasitism was highest on collard，followed by eggplant，cucumber and tomato in order of height，regardless the nymphal stage of whitefly that was parasitized. On a certain plant，*E. bimaculata* reduced its parasitization on the 1st or 2nd instars when the 3rd and 4th instars were available，so parasitisms on 1st and 2nd instars in single instar experiments were higher than that in multiple instar experiments，*e.g.* 18.30 *vs.* 10.74 on collard on 1st instar，while parasitisms on 3rd and 4th instars in single instar experiments were lower than that in multiple instar experiments，*e.g.* 26.45 *vs.* 33.45 on eggplant on 3rd instars. All the results indicated that *E. bimaculata* preferred the 3rd instar whitefly nymphs，followed by the 4th instar，and parasitism on the 1st and 2nd instar nymphs were lower than those on the 3rd and 4th instars. In present study，*E. bimaculata* demonstrated a very systematic switch on its oviposition preference，as shown by its performance in single and multiple host instar experiments.

Table 1 Parasitisms of *Encarsia bimaculata* on *Bemisia tabaci* nymphs in single and multiple instar experiments on 4 host plants

Nymphal stage	Experiment type	Collard	Eggplant	Cucumber	Tomato
1st instar	Single instar	18.30 ± 1.60 a	15.62 ± 0.92 a	15.24 ± 1.59 a	14.34 ± 0.79 a
	Multiple instars	10.74 ± 0.46 b	11.85 ± 1.33 b	13.65 ± 1.62 b	12.27 ± 0.41 b
2nd instar	Single instar	22.11 ± 1.00 a	20.53 ± 0.75 a	18.17 ± 0.54 a	15.69 ± 0.88a
	Multiple instars	13.88 ± 1.31 b	15.66 ± 0.76 b	14.77 ± 0.29 b	13.39 ± 0.92 b
3rd instar	Single instar	31.36 ± 0.81 b	26.45 ± 1.10 b	23.17 ± 1.81 b	19.67 ± 1.31 b
	Multiple instars	38.03 ± 1.18 a	33.45 ± 1.39 a	28.50 ± 0.86 a	23.67 ± 1.52 a
4th instar	Single instar	24.21 ± 1.55 b	22.35 ± 0.33 b	18.35 ± 1.40 b	16.28 ± 0.90 b
	Multiple instars	34.54 ± 0.66 a	30.58 ± 0.90 a	23.35 ± 1.07 a	20.29 ± 0.83 a

Notes：Means with different letters within a same instar in column are significantly different at $\alpha < 0.05$ in different experiment types using Student-Newman-Kuels Multiple range test. On collard，$F = 13.69$，$df = 5, 54$，$P < 0.001$ for 1st instar；$F = 21.95$，$df = 5, 54$，$P < 0.001$ for 2nd instar；$F = 18.65$，$df = 5, 54$，$P < 0.001$ for 3rd instar and $F = 44.08$，$df = 5, 54$，$P < 0.001$ for 4th instar. On eggplant，$F = 44.52$，$df = 5, 54$，$P < 0.001$ for 1st instar；$F = 16.50$，$df = 5, 54$，$P < 0.001$ for 2nd instar；$F = 29.63$，$df = 5, 54$，$P < 0.001$ for 3rd instar and $F = 20.96$，$df = 5, 54$，$P < 0.001$ for 4th instar. On cucumber，$F = 15.85$，$df = 5, 54$，$P < 0.001$ for 1st instar；$F = 24.90$，$df = 5, 54$，$P < 0.001$ for 2nd instar；$F = 35.35$，$df = 5, 54$，$P < 0.001$ for 3rd instar and $F = 31.23$，$df = 5, 54$，$P < 0.001$ for 4th instar. On tomato，$F = 11.52$，$df = 5, 54$，$P < 0.001$ for 1st instar；$F = 18.35$，$df = 5, 54$，$P < 0.001$ for 2nd instar；$F = 16.34$，$df = 5, 54$，$P < 0.001$ for 3rd instar and $F = 16.58$，$df = 5, 54$，$P < 0.001$ for 4th instar.

Given all instars of *B. tabaci* nymphs，*E. bimaculata* reduced its oviposition on the 1st and 2nd instars，and increased the parasitism on the 3rd and 4th instars on all the 4 plants（Table 1）. The largest change were observed on collard，increasing with a value of 10.33% on the 4th instar nymph when the parasitisms between single and multiple host instar experiments were compared，followed by that on eggplant，increasing 8.23% on the 4th instar nymph，and on collard decreasing 8.23% on the 2nd instar nymph；the smallest change was observed on cucumber，parasitism decreased 1.59% on the 1st instar nymph（Fig. 1）. The results indicated that *E. bimaculata* is easy to encounter or access more suitable instar nymphs on glabrous plants or those with few hairs than on hirsute plants，such as tomato.

The oviposition of *Er.* sp. nr. *furuhashii* on 4 instar nymphs of *B. tabaci* in single and multiple instar experiments was showed in Table 2. Although to a certain stage，the oviposition preferences of *Er.* sp. nr. *furuhashii* on the 4 host plants were consistent to those of *E. bimaculata*，but the changes were partly different to the latter when the results in single and multiple experiments were compared. When all the 4 instars were available，*Er.* sp. nr. *furuhashii* reduced its parasitization on the 1st，2nd and 4th instars on collard and eggplant，while only reduced its parasitization on the 1st and 4th but not 2nd instars on cucumber and tomato. All the results indicated that *Er.* sp. nr. *furuhashii* preferred the 3rd instar whitely nymphs，followed by the 2nd instars，and parasitism on the 1st and 4th instar nymphs were lower than those on the 3rd and 2nd instars.

When give all instars nymphs，the parasitism change of *Er.* sp. nr. *furuhashii* on collard was generally the largest，reducing 10.38% on the 1st instar nymphs when the parasitisms in single and multiple experiments were compared，and the smallest was observed on tomato，increasing 1.10% on the 3rd instar nymph（Fig. 2）.

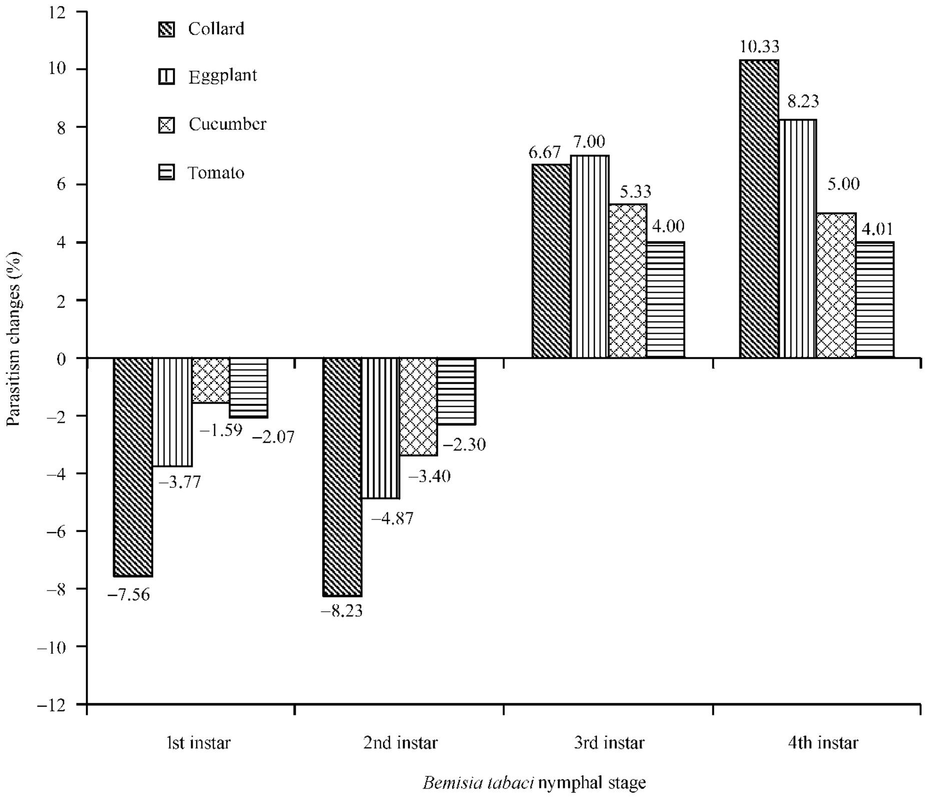

Fig. 1　Changes of *Encarsia bimaculata* parasitism on whitefly nymph in different instars as nymphs in four instars were given simultaneously to the parasitoid when compared to those when exposed only one instar of *Bemisia tabaci* at a time to parasitoid

Table 2　Parasitisms of *Eretmocerus* sp. nr. *furuhashii* on *Bemisia tabaci* nymphs in single and multiple instar experiments on four host plants

Nymphal stage	Experiment type	Collard	Eggplant	Cucumber	Tomato
1st instar	Single instar	28.18 ± 0.87 a	23.00 ± 1.25 a	22.06 ± 0.84 a	18.16 ± 0.96 a
	Multiple instars	17.80 ± 1.88 b	15.56 ± 1.51 b	15.11 ± 1.62 b	14.54 ± 1.79 a
2nd instar	Single instar	34.67 ± 0.73 a	29.41 ± 2.05 a	25.36 ± 0.97 a	21.85 ± 1.42 a
	Multiple instars	26.46 ± 2.49 b	28.13 ± 2.20 a	28.13 ± 2.83 a	23.31 ± 2.10 a
3rd instar	Single instar	49.34 ± 1.85 a	41.72 ± 1.31 a	35.48 ± 0.49 b	33.64 ± 1.18 b
	Multiple instars	51.13 ± 2.92 a	43.47 ± 2.05 a	39.43 ± 2.36 a	38.84 ± 2.51 a
4th instar	Single instar	20.17 ± 1.42 a	16.52 ± 1.02 a	15.50 ± 0.55 a	16.94 ± 1.44 a
	Multiple instars	14.71 ± 1.54 b	14.66 ± 1.93 a	12.99 ± 1.80 a	14.13 ± 1.97 a

Notes：Means with different letters within a same instar in column are significantly different at $\alpha < 0.05$ in different experiment types using Student-Newman-Kuels Multiple range test. On collard，$F = 153.66$，$df = 5, 54$，$P < 0.001$ for 1st instar；$F = 74.20$，$df = 5, 54$，$P < 0.001$ for 2nd instar；$F = 3.52$，$df = 5, 54$，$P = 0.092$ for 3rd instar and $F = 60.25$，$df = 5, 54$，$P < 0.001$ for 4th instar. On eggplant，$F = 33.61$，$df = 5, 54$，$P < 0.001$ for 1st instar；$F = 6.30$，$df = 5, 54$，$P = 0.106$ for 2nd instar；$F = 2.38$，$df = 5, 54$，$P = 0.071$ for 3rd instar and $F = 2.56$，$df = 5, 54$，$P = 0.068$ for 4th instar. On cucumber，$F = 15.25$，$df = 5, 54$，$P = 0.006$ for 1st instar；$F = 4.95$，$df = 5, 54$，$P = 0.226$ for 2nd instar；$F = 42.31$，$df = 5, 54$，$P < 0.001$ for 3rd instar and $F = 5.31$，$df = 5, 54$，$P = 0.084$ for 4th instar. On tomato，$F = 3.26$，$df = 5, 54$，$P = 0.886$ for 1st instar；$F = 2.82$，$df = 5, 54$，$P = 0.095$ for 2nd instar；$F = 19.37$，$df = 5, 54$，$P = 0.003$ for 3rd instar and $F = 4.16$，$df = 5, 54$，$P = 0.076$ for 4th instar.

4　DISCUSSION

The host instar preference of *E*. *bimaculata* and *Er*. sp. nr. *furuhashii* had not been reported before，but some other species of *Eretmocerus* spp. and *Encarsia* spp. have been studied (Gerling，1966；Nechols and Tauber，1977；Vet and van Lenteren，1981；Headrick *et al*.，1995，1996). As to the mechanism of the host instar preference in parasitic aphelinids，there are two different hypotheses. One is inclined to the younger instars be preferred. Gerling (1990) suggested that younger host instars (the 1st and 2nd instars) are less able to defend themselves with horizontally protruding wax filaments as compared to the older instars (the 3rd or especially 4th instars)，as a result the aphelinids preferred the younger instars. However，another hypothesis is based on the older

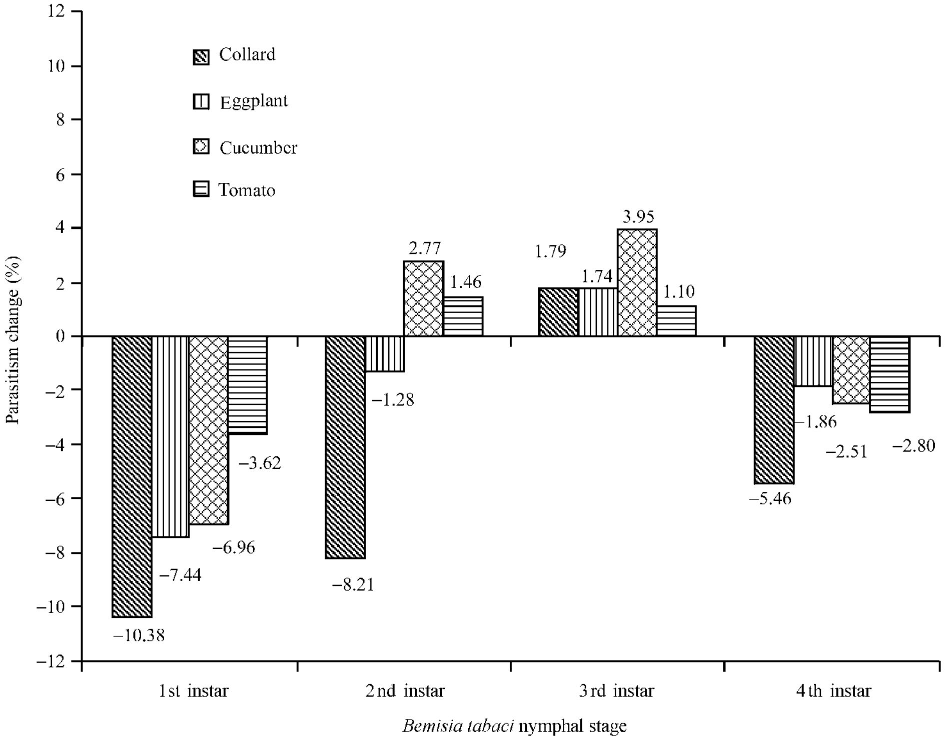

Fig. 2 Changes of *Eretmocerus* sp. nr. *furuhashii* parasitism on whitefly nymph in different instars as nymphs in four instars were given simultaneously to the parasitoid compared to those when exposed only one instar of *Bemisia tabaci* at a time to parasitoid

instars to be preferred. Liu and Stansly (1996) suggested that larger hosts could provide immediate resources for larval development, thereby maximizing intrinsic rate of increasing (r_m) though decreasing generation time, increasing fecundity, or both. It sounds that the results in our study support the second hypothesis, as an endoparasitoid, *E*. *bimaculata* preferred the older host instars, and the developmental time on them were shorter than those on younger instars where the fecundities were higher, and the mortality (not shown in this report) were lower on the older host instars. The host instar preference of *Er*. sp. nr. *furuhashii* was also consistent with the second hypothesis although it apparently preferred the 2nd and 3rd host instars. As an ecto-endoparasitoid, *Er*. sp. nr. *furuhashii* as well as other *Eretmocerus* parasitoids deposit their eggs underneath whitefly nymphs on the leaf surface, and the 1st instar larvae enter the host by penetrating the underside of the host upon eclosion (Foltyn and Gerling, 1985). The parasitoid eggs required an average time of approximately 3 days to hatch. During this period, the 2nd and 3rd instar hosts that *Eretmocerus* parasitoids preferred would already develop to the 3rd and 4th instars, respectively. Thus *Eretmocerus* parasitoids prefer to use the nutrition of older instar hosts to complete their development in fact, which could be beneficial their development, survival and reproduction of the population in nature.

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两种蚜小蜂对烟粉虱寄主的产卵选择性

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摘要：双斑恩蚜小蜂和桨角蚜小蜂是华南地区烟粉虱的两种优势种寄生蜂。本文研究了两种寄生蜂对不同龄期烟粉虱寄主的产卵选择特性。结果表明：两种寄生蜂均可寄生烟粉虱的1～4龄若虫。当只有1个龄期的烟粉虱若虫存在时，双斑恩蚜小蜂更多地寄生3龄和4龄若虫，较少寄生1龄和2龄若虫；而桨角蚜小蜂则更多地寄生3龄和2龄若虫，较少寄生1龄和4龄若虫。在4个龄期若虫同时存在时，双斑恩蚜小蜂会明显增加对3龄和4龄若虫的产卵寄生，降低对1龄和2龄若虫的寄生；而桨角蚜小蜂则增加对2龄和3龄若虫的寄生，减少对1龄和4龄若虫的寄生。寄主植物的差异不影响这二种蚜小蜂对各龄期烟粉虱若虫的产卵选择倾向。结果提示，烟粉虱若虫3龄和4龄是双斑恩蚜小蜂最适宜的寄主，而桨角蚜小蜂最适宜的寄主是烟粉虱2龄和3龄若虫。

关键词：双斑恩蚜小蜂；桨角蚜小蜂；烟粉虱；产卵选择性；生物防治

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